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New information about the pre-imaginal morphology of genus *Graptomyza* (Diptera, Syrphidae, Volucellini): description of third-instar larva and re-description of puparium of *G. signata* (Walker, 1860)

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Abstract

Pre-imaginal morphology of the flower fly species *Graptomyza signata* (Walker) is described and figured in detail based on specimens collected on a decomposed *Aloe*-like plant in KwaZulu-Natal province, South Africa. Third-instar larva is described for the first time and the puparium morphology is re-described using both light (optical) and electron microscopy. The present work represents the second larval description for a species of the genus *Graptomyza*, after the description of the larva of *G. alabeta* Séguy. The immatures of these two *Graptomyza* species were examined and compared to the pre-imaginal stages of the other members of the tribe Volucellini, pointing out the possible diagnostic characters of the genus *Graptomyza*. Moreover, new DNA barcodes are provided for *G. signata* and deposited in the NCBI GenBank.

Keywords

Graptomyza, Volucellini, flower flies, hoverflies, DNA barcoding, larval morphology, immatures

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Introduction

Graptomyza Wiedemann, 1820 (Diptera, Syrphidae) is a medium-size flower fly genus with 90 described valid species, with several more still undescribed, widespread in the Afrotropical, Oriental and Australasian Regions, including the Pacific (Thompson et al. 2017). *Graptomyza* is a member of the tribe Volucellini (subfamily Eristalinae), which further comprises the genera *Volucella* Geoffroy, 1762, *Ornidia* Lepeletier & Serville, 1828 and *Copestylum* Macquart, 1846. This tribe is characterised by having the antennal arista plumose or pectinate and vein M_1 straight or recessive (Thompson 1972), with some exceptions (Hull 1949, Thompson 1972, 1981, 1991). The tribe was shown to be monophyletic using adult morphological characteristics (Thompson 1972, 1991, Whittington 1992, Hippa and Ståhls 2005), molecular characters (Young et al. 2016) or a combination of both (Ståhls et al. 2003, Mengual et al. 2015). Nevertheless, the intratribal relationships are not yet well understood (Thompson 1972).

In the Afrotropical Region, 19 *Graptomyza* species are known and some new species await formal description (Whittington 1992, 1994a). Based on the distributional range (tropical and subtropical environments in Africa) and biological data, Whittington (1994a) stated that most Afrotropical *Graptomyza* species are forest inhabitants as no records from arid regions are known. Nevertheless, some species are found in *Acacia*-savannah habitats or montane grasslands and even *Graptomyza signata* (Walker, 1860) is distributed across tropical dry savannah (Whittington 1992, 1994a). Larvae of this genus have saprophagous feeding habits (Thompson 1991, Whittington 1994a). While little is known about the ecology of the adults, Hull (1949: 350) suggested a resemblance between *Graptomyza* imagoes and stingless bees (Hymenoptera, Apidae, Meliponini), but Whittington (1992) questioned this mimicry.

Most of the information of the genus is based on the analysis of the morphology and biology of adults, as very little is known about the pre-imaginal stages. Amongst all known *Graptomyza* species, immature stages have been described only for four species. Whittington (1994b) described the puparium of an Australian species, *Graptomyza mitis* Curran & Bryan, 1926 and the puparia of two Afrotropical taxa, *G. signata* and *G. triangulifera* (Bigot, 1883). Later, Krivosheina and Krivosheina (1996) described the puparium of *G. alabeta* Séguy, 1948 from material collected in the Russian Far East.

Graptomyza signata belongs to the *varia* species group (Whittington 1992) and is characterised by strong setae on metatibia and dark chevron markings on the abdomen. The species is widespread in tropical and subtropical Africa (Whittington 1994a: table 1, Dirickx 1998) and it is the predominant species of the genus south of the Zambezi River (Zimbabwe, Mozambique and South Africa). The species is present during the whole year, being the Afrotropical species of this genus with the highest number of records, although its phenology has a significant decline in records during the cooler months (June to September), coinciding with the dry season (Whittington 1994a).

The main objectives of the present work are to describe for the first time the larval morphology of the third-instar larva of *G. signata* and to re-describe the morphology of the puparium, together with the cephalopharyngeal skeleton, highlighting its diagnostic features. At the same time, new DNA barcodes for the species are provided.

Materials and methods

Collecting site and adult identification

Nine larvae of *G. signata* were collected on decomposed roots and stems of an *Aloe*-like plant in a nursery garden in Queen Elizabeth Park Nature Reserve, Pietermaritzburg (KwaZulu-Natal province), South Africa, in December 2012 (29°33.981'S, 30°19.194'E; 900 m elev.). Larvae were reared with the same plant tissue in which they were found until they pupated. Emerged adults were killed by freezing and pinned for identification and preservation. For adult identification, keys from Whittington (1992, 1994a) were used and specimens were also checked against material from the reference collection of the authors.

Pre-imaginal morphological studies

Terminology used for larval and pupal descriptions follows Rotheray (1991, 1993). Only two third-instar larvae (L3) were selected for preservation and description. For permanent preservation, larvae were immersed in cold water to extend them and then heated slowly for about four minutes to kill them.

The larval integument was coated thickly in dried decaying plant tissue, particularly the basal region of the posterior respiratory process and the three pairs of lappets at the end of the body. To remove this dry layer, the larvae were immersed in water for 12 hours. After this period, with the help of a binocular microscope, larvae were cleaned using brushes of different strength and thickness. Subsequently, the larvae were washed and preserved in 70% ethyl alcohol.

Debris, adhering to the puparium integument, was removed by placing the specimens in an ultrasonic cleaner for a few minutes. Cleaned specimens were mounted on stubs and examined with a scanning electron microscope (S3000N Hitachi) using variable-pressure (or low vacuum) mode. This technique allows a direct evaluation of the specimens without coating the samples with gold. These studies were conducted in the technical research services at the University of Alicante. Cephalopharyngeal skeletons were extracted from the puparia and cleared by immersion in a 10% solution of potassium hydroxide (KOH) for 5 minutes. After the clearing process, the structures were preserved in glycerine. Third-instar larvae, puparia and cephalopharyngeal skeleton morphology were analysed using a stereomicroscope (Leica M205C) and pictures were taken using a camera adapted to it (Leica DFC450). Dimensions of preserved specimens were measured using the ImageJ informatics tool (Schneider et al. 2012) based on the pictures previously obtained.

Molecular studies

DNA was extracted from a single leg of each of the two pinned specimens (lab codes GS2 and GS3) and one puparium of *G. signata* (of GS2) using the Phire Tissue Direct PCR master Mix #F-170S kit (Thermo Scientific Baltics UAB, Vilnius, Lithuania) following the Dilution and Storage protocol with some modifications. The Phire Tissue Direct PCR master Mix is designed to perform PCR directly from tissue samples with no prior DNA purification. The tissue sample was placed in an Eppendorf tube in 30 µl of Dilution Buffer, with 0.8 µl of DNA Release Additive. The tube was briefly vortexed and centrifuged, then 1) incubated at room temperature for about 20 min, 2) placed in +56 °C for 10 min and 3) placed in a pre-heated block at 98 °C for 2 min and finally centrifuged at 11 000 rpm for 1 min. One µl of supernatant was used in a 25 µl PCR reaction using the kit PCR master Mix. The cycling conditions were: initial denaturation at 98 °C for 5 min, 40 cycles of denaturation at 98 °C for 5 s, annealing at 49 °C for 30 s, extension at 72 °C for 20 s and final extension at 72 °C for 1 min. The mtDNA COI barcode of the 5' region of COI (=COIa) was amplified with forward primer LCO1490 (5'-GCTCAACAAATCATAAAGATATTGG-3') and reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994). Amplified PCR products were electrophoresed on 1.5% agarose gels and treated with Exo-SapIT (USB Affymetrix, Ohio, USA) prior to sequencing. Both PCR primers were used for sequencing. The Big Dye Terminator Cycle Sequencing Kit (version 3.1) (Applied Biosystems, Foster City, CA, USA) was used on an ABI 3730 (Applied Biosystems, Foster City, CA, USA) genetic analyser at the Sequencing Service Laboratory of the Finnish Institute for Molecular Medicine (www.fimm.fi). The sequences were edited for base-calling errors and assembled using Sequencher (version 5.0) (Gene Codes Corporation, Ann Arbor, MI, USA).

The obtained mtDNA COI sequences were individually compared against the BOLD systems v4 (boldsystems.org, accessed 16 October 2018) and the NCBI GenBank databases, using BLASTn, for comparison. Sequences produced in this study were deposited in the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/), accession numbers MK415842 (GS2_Leg) and MK415843 (Gs3_Pupa).

Results

A total of nine third-instar larvae were collected in South Africa: KwaZulu-Natal Prov., Pietermaritzburg, Queen Elizabeth Park Nature Reserve nursery garden on 15.12.2012, by two of the authors (Snežana Radenković and Santos Rojo). Two specimens were preserved for morphological studies while the seven remaining larvae were allowed to pupate. One of the pupae did not emerge and was preserved. One of the adults emerged on 5.1.2013 (female), three of them on 6.1.2013 (one female and two males) and two on 7.1.2013 (one female and one male). Adults were also collected during December on *Euphorbia* flowers in Royal Natal National Park, KwaZulu-Natal Prov., Drakensberg Mountain by AV.

Adult identification

Examined material

Adult specimens were identified as *G. signata* and they matched very closely the characters used in the key by Whittington (1992) and the description therein. All the material is deposited in the Entomological Collection of the University of Alicante (Spain) (CEUA), currently at the Department of Environmental Sciences and Natural Resources.

Third-instar larval morphology of *Graptomyza signata*

Overall characters

Light-brown in colour. Length 6.3 ± 0.3 mm, Width 2.2 ± 0.15 mm (N = 2). Dorsoventrally flattened, broadly rounded anteriorly and slightly tapered posteriorly, with protruding breathing tube. Dorsal and lateral surface hairy, covered by long, thin and slightly sclerotised setae, increasing in size in the anal segment. Vestiture of the surface not forming a pattern of transverse rows (Fig. 1B). Ventral surface covered by setae whose length decreases from the margins to the inner side (Fig. 1A).

Cephalopharyngeal skeleton

Mandibles and mandibular lobes internal (Fig. 1C). Mandibles heavily sclerotised; elongated and curve-shaped in lateral view. Mandibular lobes attached to the mandibles; their inner surface covered by transversal ridges. Cephalopharyngeal skeleton about two times as long as wide, bar-shaped, formed by dorsal and ventral cornua and mandibular lobes. Dorsal cornu apically rounded and more sclerotised than rest of skeleton. Both clypeal sclerites are fused with labrum and reduced, representing one fifth of total length of skeleton (Fig. 1D). Tentorium extending from dorsal to ventral cornu. Cibarial chamber located in ventral cornu, with transversal ridges on bottom surface. Tentorial bar connects mandibles with remainder of cephalopharyngeal skeleton (Fig. 1E).

Pseudocephalon and thorax

Dorsal, ventral and lateral lips well developed. Lateral lips rounded and covered by a dense tuft of long and thin setae at apex, becoming thinner at base. Antenno-maxillary organs well developed, located between dorsal lip and dorsal surface of prothorax. The antenno-maxillary organs are formed by a pair of cylindrical-shaped structures, each with antenna and maxillary palpus clearly identified. Several satellite sensilla present on top of antennae and maxillary palpi. Ventral surface of anterior fold of prothorax covered by sclerotised and hook-shaped spicules with apex more sclerotised than basal part. The spicules face backwards and reach up dorsally to second sensilla of prothorax (Fig. 1C). Dorsal surface of the prothorax with three longitudinal grooves. Dorsal

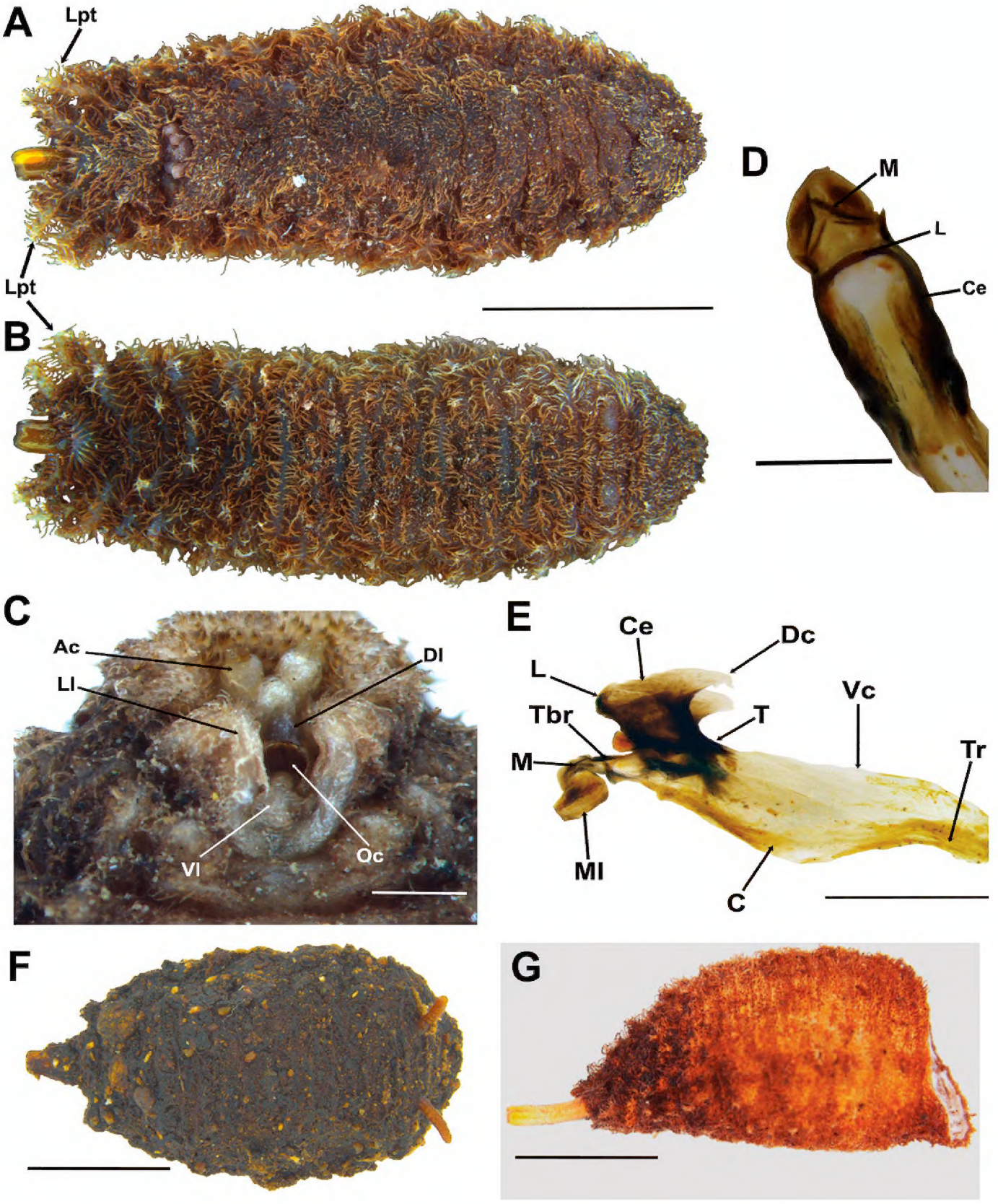

Figure 1. *Graptomyza signata* larvae and puparium. **A** larvae, ventral view **B** larvae, dorsal view **C** prothorax of larvae, ventral view **D** cephalopharyngeal skeleton, dorsal view **E** cephalopharyngeal skeleton, lateral view **F** pupae not cleaned, dorsal view **G** puparium cleaned, lateral view. Abbreviations: Ac – Antennomaxillary complex; C – Cibarium; Ce – Clypeal sclerite; Dc – Dorsal cornu; Dl – Dorsal lip; L – Labrum; Ll – Lateral lip; Lpt – Lappets; M – Mandibles; Ml – Mandibular lobes; Oc – Oral cavity; T – Tentorium; Tbr – Tentorial bar; Tr – Transversal ridges; Vc – Ventral cornu; Vl – Ventral lip. Scale bars: 2 mm (**A, B, F, G**), 0.5 mm (**C, E**), 0.25 mm (**D**).

pairs of prothoracic sensilla on small basal papillae with up to 4 apical setae. Anterior spiracles located at lateral margins of prothorax, posterior to third sensilla; cylindrical in shape and with a rounded apex, about one and a half times longer than broad. They

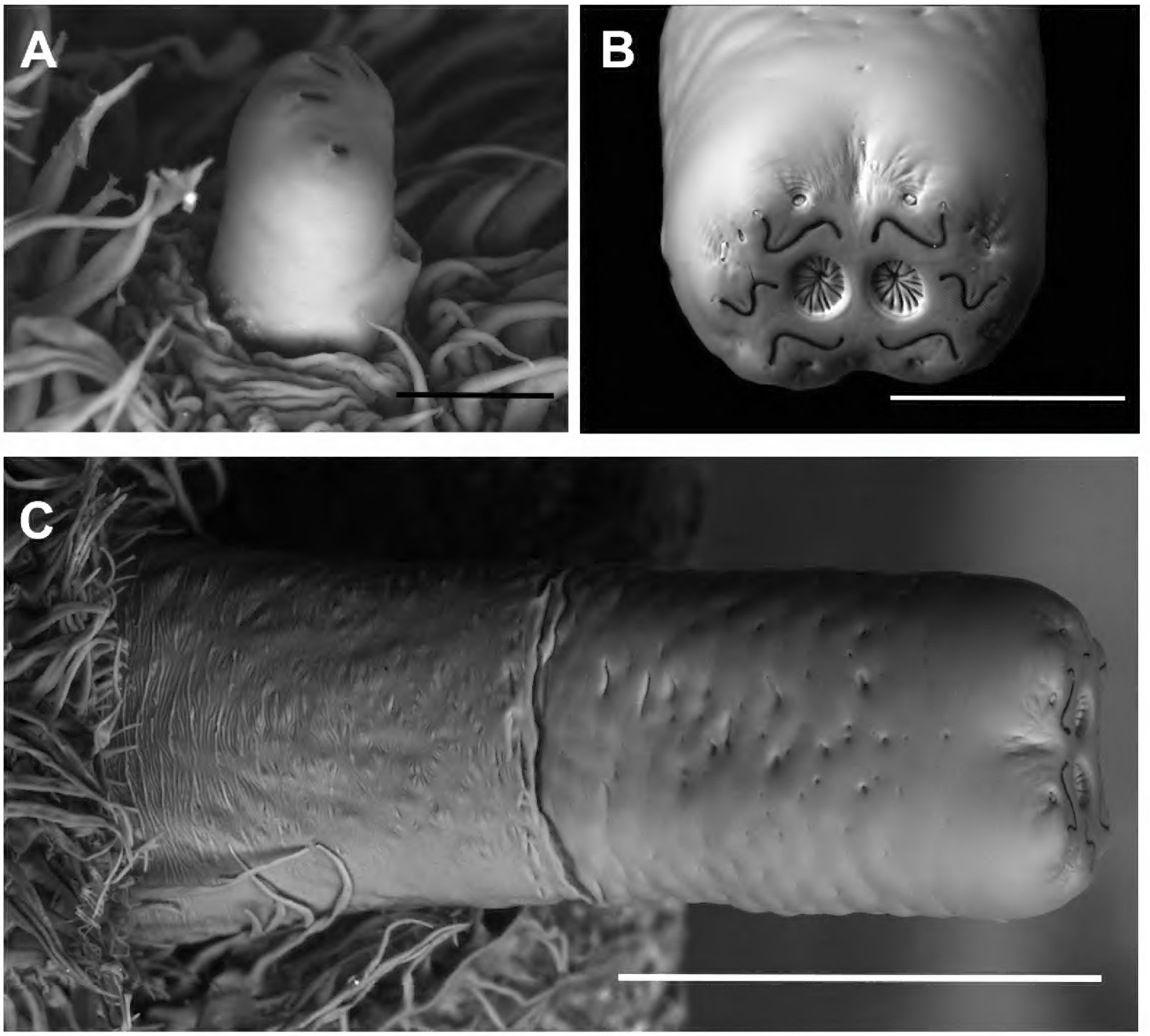

Figure 2. Spiracular morphology of *Graptomyza signata* pupa. **A** anterior larval spiracle **B** spiracular plate of the Posterior Respiratory Process **C** Posterior Respiratory Process, dorsal view. Scale bars: 50 μm (**A**), 200 μm (**B**), 500 μm (**C**).

have 3–4 elongated spiracular openings at the apex and a large scar at base, directed towards margins of the larvae (Fig. 2A). Pair of mesothoracic prolegs barely developed on ventral surface of mesothorax. Dorsal pairs of sensilla of mesothorax and metathorax located on top of small and basal papilla and dorso-lateral pairs of sensilla located on well-developed papillae with several apical setae.

Abdomen

Primordia of pupal spiracles on dorsal surface of first abdominal segment. Prolegs barely developed, represented as transversal ridges without crochets, present in abdominal segments 1–7. Abdominal segments with dorsal pairs of segmental sensilla 1, 2 and 3 with small basal papillae, increasing in size towards segment 7. Dorso-lateral pairs of sensilla 4, 5 and 6 with well-developed papillae, all surrounded by apical setae, increasing in size to-

wards the posterior end of the larva. Anal segment about one and a half times longer than 7th abdominal segment, divided in two sections: one anterior section with two pairs of dorso-lateral lappets bearing sensilla 1, 2 and 3; and one posterior section with one pair of ventral lappets with sensilla 4 at the base and sensilla 5 on the apex. First lappet slightly shorter (± 400–450 μm) than second and third lappets (± 500–550 μm), fully covered with long and thin setae and facing the posterior margin of the larvae (Fig. 1A, B).

Posterior respiratory process

Shiny, light brown to reddish in colour, sub-elliptical in cross section, length 1.0 ± 0.25 mm (2.5 times as long as wide). The length of the posterior respiratory process (prp) varies between larva and pupa due to the larval tissue partially covering the surface and the pupa with all this tissue retracted, exposing the entirety of the structure. In the middle of its length, there is a shallow transverse ridge. The ornamentation from base to transversal ridge presents faint transversal wrinkles more visible at base. The shape of apex (from transversal ridge to spiracular plate) is rectangular, with irregular spaced dents in basal three quarters and being smooth in apical quarter (Fig. 2C). Spiracular plate presents three pairs of curved openings around two central round scars and four pairs of branched inter-spiracular setae (Fig. 2B).

Chaetotaxy

Prothorax (P) with 11 pairs of sensilla, mesothorax (Ms) and metathorax (Mt) with eight pairs of sensilla. Abdominal segments 1–7 with nine pairs of sensilla, anal segment (A8) with eight pairs of sensilla and three pairs of lappets (Fig. 3).

Puparium of *Graptomyza signata*

Overall description

Ovate; dorsally gibbose; sub-cylindrical in cross-section. Anterior end truncated, tapered posteriorly and flattened ventrally. Dull, light brown to reddish in colour. Surface covered with dense pubescence, longer in posterior segments. Anal segment with three pairs of lappets. Rough integument with larval segmentation persisting as transverse folds and wrinkles (Fig. 1G). Length including prp: 7.0 ± 1.25 mm; width: 3.0 ± 0.5 mm; height: 2.5 ± 0.5 mm (N=5).

Pupal spiracles

Sub-cylindrical structures of 0.94 ± 0.03 mm long and 0.14 ± 0.02 mm wide (N=4), curved backwards and with the end slightly tapered. Shiny, light brown to

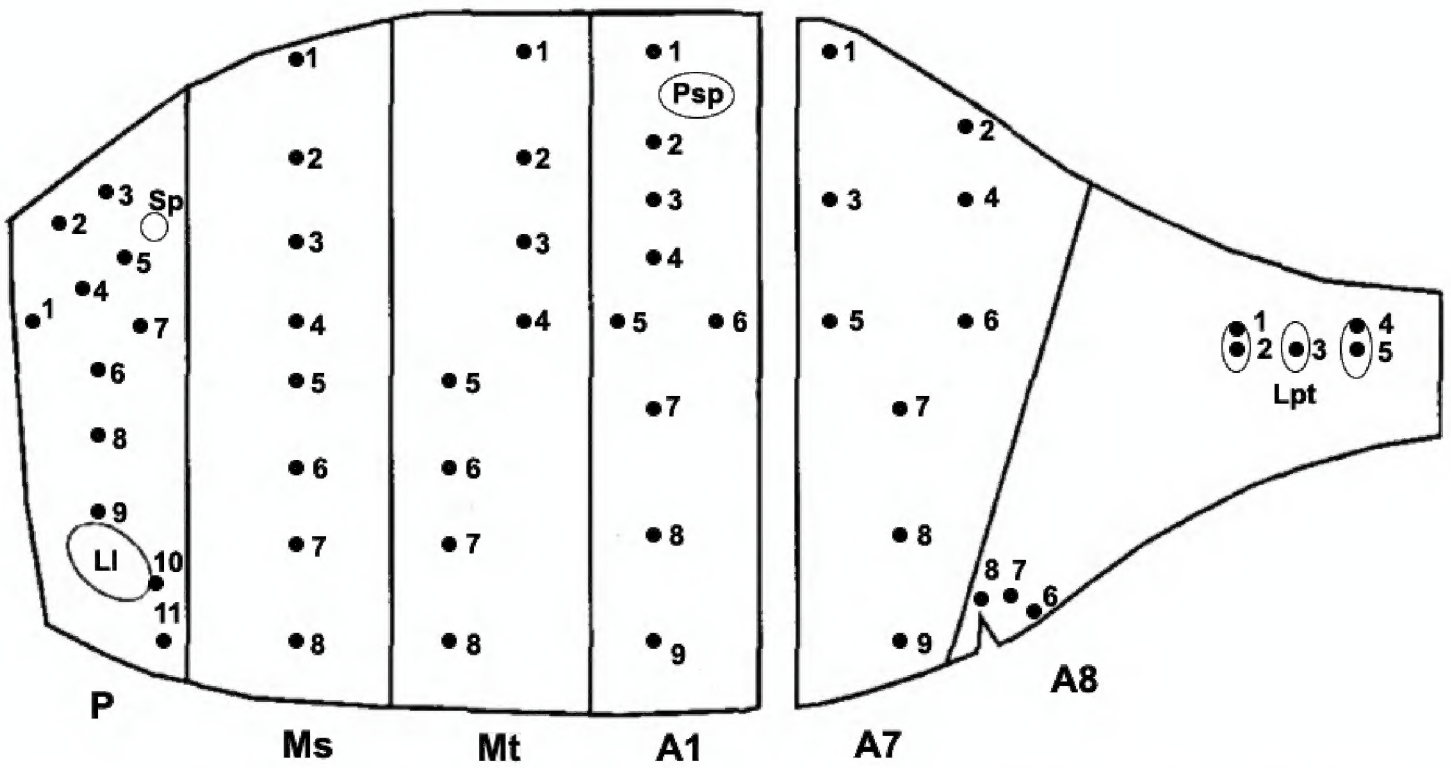

Figure 3. Map of the chaetotaxy of the third instar larva of *Graptomyza signata* in lateral view showing the positions of the sensilla group. P – Prothorax, Ms – Mesothorax, Mt – Metathorax, A1–A7 – Abdominal segments, A8 – Anal segment. Sp – Anterior spiracle, Ll – Lateral lip, Psp – Primordia of pupal spiracles, Lpt – Lappets.

reddish. Approximately 80% of dorsal and lateral surface is covered with irregular spaced and oval to sub-elliptical tubercles which are barely prominent (Fig. 4A). These tubercles are arranged in 7–8 lateral bands that are dorsally less prominent (Fig. 4B). Each tubercle bears 1–3 oval spiracular openings accompanied by a keel shaped structure. The entire surface, including the space between tubercles is reticulated with a polygonal pattern. At the centre of some of these polygons appears a very small hole or aperture (Fig. 4C). Both spiracles are projected from the middle of the upper part of the operculum, separated by a distance about the length of one spiracle (Fig. 1F).

MtDNA COI barcodes

Three COI sequences were obtained from two raised adults and one from one of the puparia, the length of the sequences varying between 563 and 660 bp. There was only a single nucleotide difference between the two adult barcodes (99.8% similarity). The barcode of the adult and of its puparium were similar, except for two ambiguous base positions due to the lower quality of the DNA barcode from the puparium. The GenBank BLASTn query returned five *Graptomyza* barcodes with the highest similarities (≥ 91%). BOLD query results were similar, but yielded an additional two highly similar barcodes (≥ 98%) of unnamed and unpublished specimens.

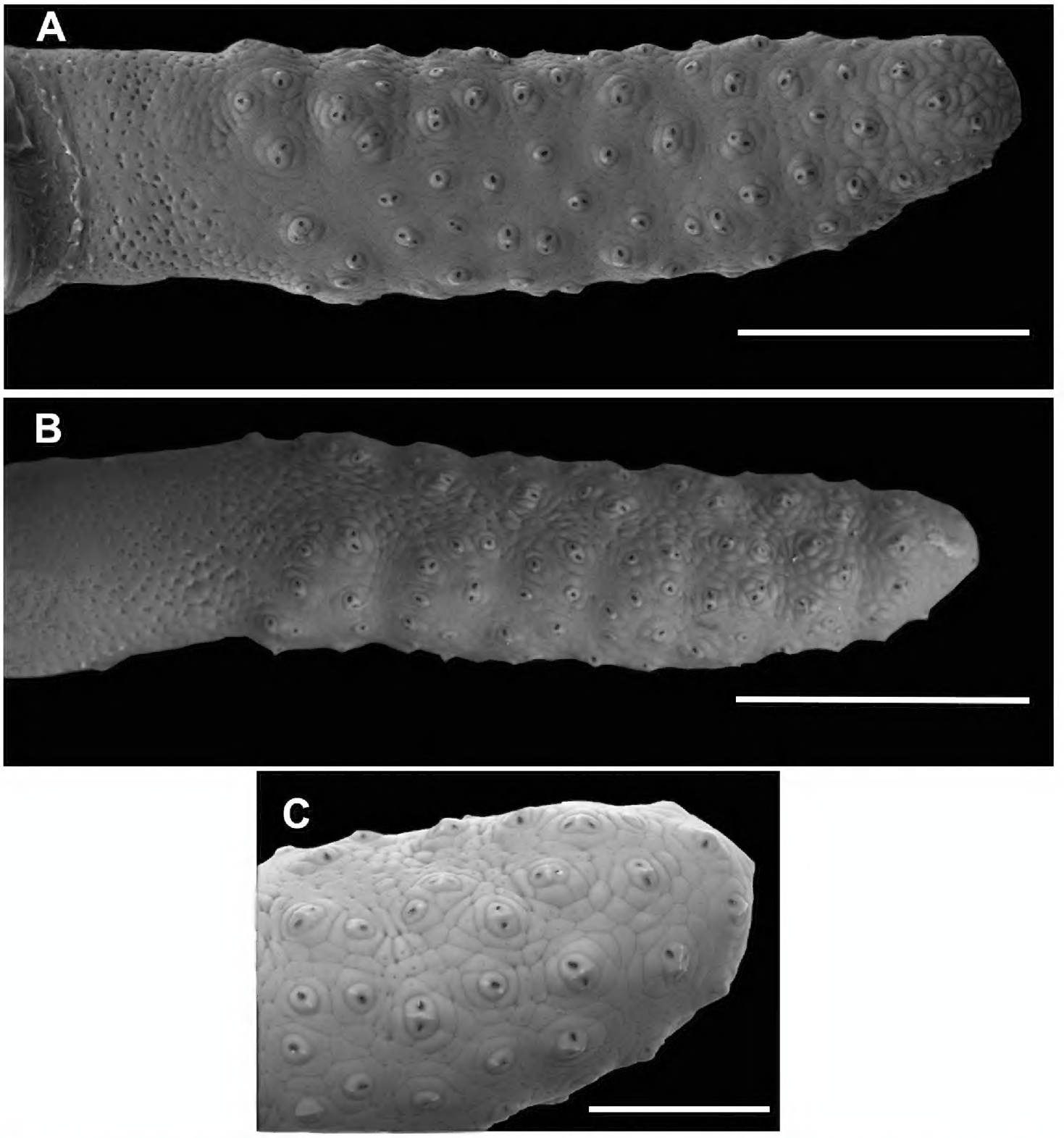

Figure 4. *Graptomyza signata* pupal spiracles. **A** pupal spiracle, dorsal view **B** pupal spiracle, ventral view **C** spiracular openings of pupal spiracles. Scale bars: 0.25 mm (**A, B**), 0.01 mm (**C**).

Discussion

Whittington (1994b) described and illustrated the puparium, cephalopharyngeal skeleton and posterior spiracles of *G. signata*, but specimens from the present study showed some differences in comparison, specifically in the shape and surface of the puparium. Whittington defined it as "*finely lobate*" and "*finely setose*", with the provided illustrations (Whittington 1994b) showing a smooth puparium with no clear vestiture. The puparia used in the present study were densely covered by setae, especially in the posterior apex, with reminiscences of the larval tegument, such as transverse folds and wrinkles. The lappets were preserved as well (Fig. 1G). The current study specimens

were also fully covered with dried decaying plant tissue and soil right after its sampling and the overall appearance (Fig. 1F) was very similar to the illustrations of Whittington (1994b). Taking this into account and considering that Whittington (1994b) stated that his examined specimens were described without being cleaned, it can be assumed that the puparium described by Whittington (1994b) was covered with dried decaying plant tissue and soil, thus preventing a proper description.

The puparium of *G. signata* is very similar to the puparium of the three other *Graptomyza* species for which the puparium has been described. They all share a more or less developed setose vestiture and the presence of three pairs of lappets (unclear in *G. mitis*). The edge of the spiracular plates is more defined and irregular in *G. alabeta*, *G. triangulifera* and *G. mitis* than in *G. signata*. The shape of the spiracular openings differs between the four species: they are straight and run almost parallel in *G. alabeta* (Fig. 5A, B), are V-shaped in *G. mitis*, slightly curved in *G. triangulifera* and convoluted in *G. signata* (Fig. 2B). The shape of the pupal spiracles is annulated, or slightly annulated, in the four species. The ornamentation of the pupal spiracles was studied in *G. signata* and in *G. alabeta* and it was found that the annulated design is due to the presence of several bands of sub-elliptical tubercles bearing 1–3 (*G. signata*) and 2–5 (*G. alabeta*) oval spiracular openings accompanied by a keel shaped structure (Fig. 5E); these bands are more obvious in *G. signata*. The surface of the pupal spiracles is reticulated with a polygonal pattern: in *G. alabeta*, one small hair is present on each polygon in the area shared with the spiracular openings (Fig. 5D, E), while *G. signata* has a hole in some of the polygons (Fig. 4C).

Krivosheina and Krivosheina (1996) published the only larval description of the genus *Graptomyza* so far and kindly lent the authors some of the *G. alabeta* material used in their original description. Pre-imaginal stages of both species are very similar in general morphology. The general vestiture is made by long and thin setae with no apparent distribution pattern, although the vestiture is denser and has less sclerotised setae in *G. signata*, from which its hairy appearance can be attributed. In both species, the thoracic and abdominal sensilla are mounted in fleshy projections with apical setae surrounding it, becoming longer towards the posterior end. The anal segment is divided dorsally in two regions: an anterior region with two pairs of dorso-lateral fleshy lappets and a posterior region with one pair of ventral lappets. Larval spiracles of both species have a similar morphology. The posterior respiratory process of *G. alabeta* becomes narrower at the apex and it does not show an apparent transverse ridge (Fig. 5C), but it has an ornamented base and smooth apex, as in *G. signata*.

Known morphology of the larvae and puparia of *Graptomyza* share the diagnostic characteristics of Vollucellini, proposed by Rotheray et al. (2005): mouthparts with the mandibular lobes attached to a reduced mandibular sclerite not protruding out of the mouth and the anal segment divided into two sections with an anterior section bearing two pairs of lappets, of which the first pair has an extra lateral sensilla and a posterior section with one pair of lappets. Although the description of more immature stages of *Graptomyza* are necessary to state a more general definition of the pre-imaginal stages of the genus, the following preliminary general diagnosis for *Graptomyza* larvae is proposed: three pairs of about equally sized lappets coated by a very long vestiture, prolegs poorly developed

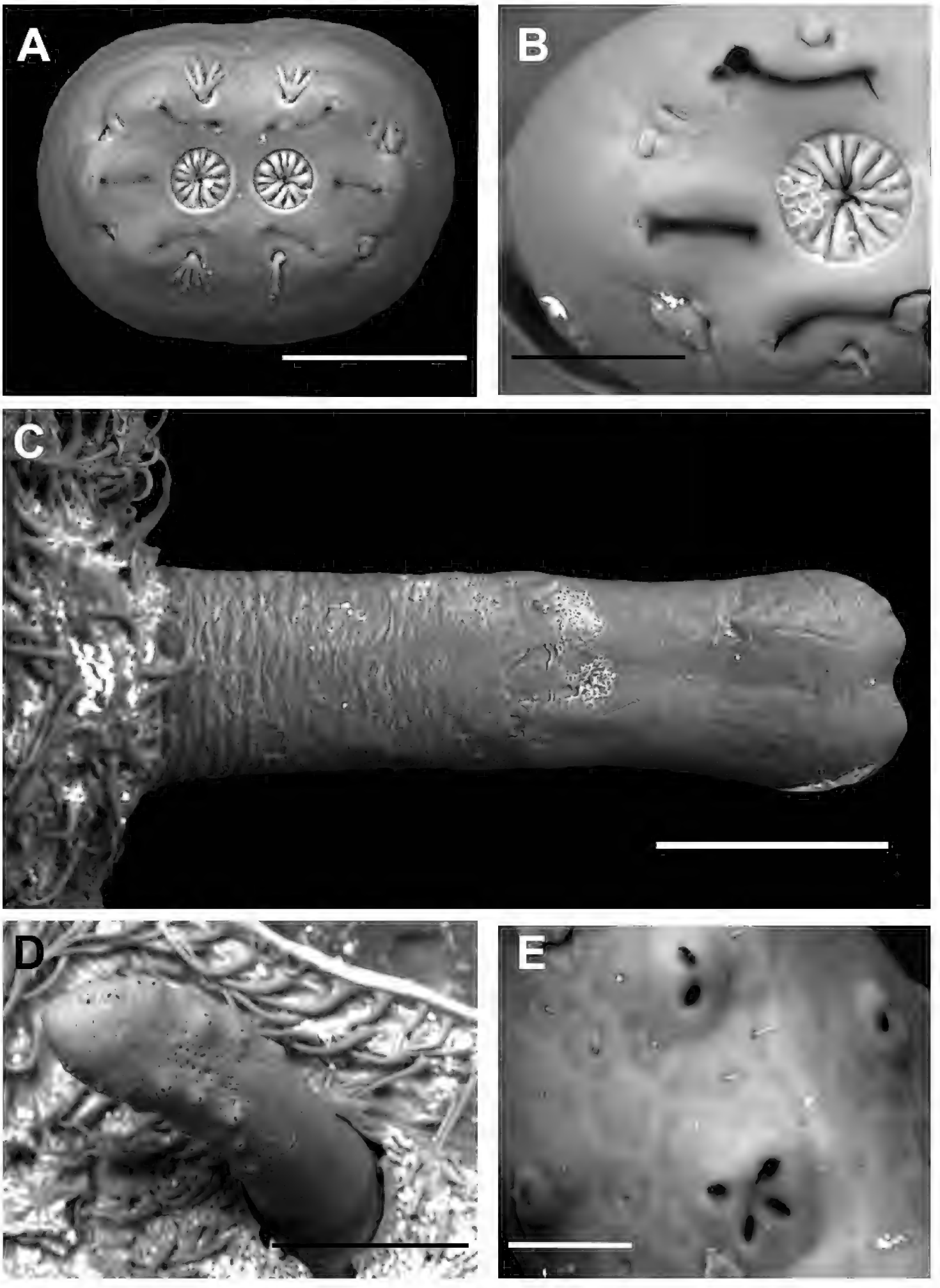

Figure 5. *Graptomyza alabeta* spiracles. **A** spiracular plate of Posterior Respiratory Process **B** detail of spiracular openings of spiracular plate of the Posterior Respiratory Process **C** Posterior Respiratory Process, dorsal view **D** pupal spiracle **E** detail of spiracular openings of pupal spiracle. Scale bars: 100 µm (**A**), 50 µm (**B**), 250 µm (**C**), 200 µm (**D**), 25 µm (**E**).

(few crochets may be discernible) or transformed into transversal ridges, anterior spiracles present, patch of spicules next to anus absent, sides of body with gradually elongating projection and ornamentation of the pupal spiracles consisting of tubercles arranged in 7–8 bands, with oval spiracular openings accompanied by a keel-shaped structure.

Whittington and Rotheray (1997) affirmed that all volucelline larvae share morphological characters associated with locomotory organs, such as broader than long prolegs with transverse rows of crochets. Nevertheless, this degree of development in the locomotory organs has not been found in *Volucella inflata* (Fabricius, 1794) (Rotheray 1999). This could be the reason why he did not include the high level of development of the locomotory organs as a diagnostic character of the tribe Volucellini in a later work (Rotheray et al. 2005). In fact, the immatures of the two *Graptomyza* species described up to now have had poorly developed prolegs without crochets. Whittington and Rotheray (1997) also observed that the feeding channel and associated structures of the volucellines are poorly developed, so that the larval thorax and trophic structure are similar to those of other filter-feeding Syrphidae. Based on current evidence, *Graptomyza* immatures are known to have saprophagous-like habits and feed on substrata rich in microorganisms. They have been reported from bark and roots of fallen trees, seed pods, tree sap and on fallen or rotting fruits, feeding on the moist substrate (Whittington 1992, 1994a; Krivosheina and Krivosheina 1996). Larvae of *G. signata* have been reported from tomato and unspecified rotting fruits (Whittington, 1994b) and from decaying roots and stems of an *Aloe*-like plant (present study).

The morphology of the cephalopharyngeal skeleton between the five species described varies in the size of the mandibles and mandibular lobes, the relative lengths of the dorsal and ventral cornua and the degree of sclerotisation of the clypeal sclerite. Dorsal cornu is generally rounded apically, always shorter than the ventral cornu. The puparium of *G. triangulifera* has the ventral cornu clearly shorter than the rest of described species.

Graptomyza larval morphology is very similar to the overall morphology of *Brachyopa* (Syrphidae, Eristalinae, Brachyopini) larvae, although they are not phylogenetically closely related (Mengual et al. 2015; Pérez-Bañón et al. 2016; Young et al. 2016). Shatalkin (1975) also compared the male genitalia between volucellines and brachyopines, although he argued that the morphological similarity was due to convergence. Krivosheina and Krivosheina (1996) also compared the larvae of *G. alabeta* with the larvae of *Brachyopa* in terms of body form, the distributional pattern of papillae, posterior respiratory process and cuticular structures. *Brachyopa* larvae occur in sap runs or in accumulations of decaying sap under bark, probably feeding on microrganisms (Rotheray 1993; Rotheray and Gilbert 1999). Thus, we consider that this similarity between larvae of *Brachyopa* and *Graptomyza* may be due to similar morphological adaptations to feed on similar media, as the sticky consistence of the decaying tissues of the *Aloe*-like plant may be very much like that of the exuding tree sap.

Descriptions of immature stages and rearing data can contribute to the systematics of a group as useful taxonomic information to develop a sound classification and a

likely evolutionary scenario (Whittington 1994b). Larval characters alone have been used to infer phylogenies in Syrphidae (Rotheray and Gilbert 1989, 1999) or higher taxonomic groups (Rotheray and Gilbert 2008), but also in combination with other types of characters, such as adult morphology and molecular characters (Ståhls et al. 2003; Mengual 2008). In a very broad DNA barcoding study on Afrotropical flower flies, Jordaens et al. (2015) reported high intraspecific K2P divergences in several putative nominal species and, amongst them, *Graptomyza triangulifera* with differences of 4.1% between studied specimens. Such observations may reflect geographical structuring or evolutionary history, as suggested by Jordaens et al. (2015) or may represent recently diverged species. We believe that the study of the ecology and morphology of pre-imaginal stages can contribute to systematics of Syrphidae, providing evidence to recognise different taxa.

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